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PATENT

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5/26/04

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE*In re Application of:*

Robert Michael ROBERTS Ex
 Jonathan Andrew GREEN and
 Sancai XIE

Serial No.: 09/273,164

Filed: March 19, 1999

For: COMPOSITIONS AND METHODS FOR
 EARLY PREGNANCY DIAGNOSIS

Group Art Unit: 1643

Examiner: C. Chen

Atty. Dkt. No.: UVMO:003

CERTIFICATE OF MAILING
37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below:

Date

Robert E. Hanson

DECLARATION OF JONATHAN A. GREEN UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
 Washington, D.C. 20231

I, JONATHAN A. GREEN, HEREBY DECLARE AS FOLLOWS:

1. I am a co-inventor of the subject matter disclosed and claimed in the above-referenced patent application.
2. I am currently employed by The University of Missouri as an Assistant Professor. I hold a Ph.D. in Biochemistry from the University of Missouri. I have been conducting research in the area of biochemistry and reproductive biology since 1991.

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3. I previously supplied a Declaration in this patent application submitting data demonstrating the isolation and use of monoclonal antibodies that detect PAGs disclosed in the above-referenced patent application. The data presented at that time demonstrated that PAGs 4, 6, 7, 16 and 20 are absent about two-months post-partum and that antibodies for these PAGs may be used in assays for the detection of pregnant bovine animals. I am submitting this Declaration to present further data obtained since the time of the studies described in my previous Declaration. This data demonstrates that, in addition to the PAGs listed above, PAG 17 and PAG 21 are also undetectable at about two months post-partum.

4. *Identification of PAGs bound by L4, A6 and J2 monoclonal antibodies.*

The isolation of monoclonal antibodies L4, A6, and J2 was as described in my previous Declaration. Further studies were carried out under my supervision to identify the PAGs detected by these antibodies as follows:

One mg of each purified mAb was first crosslinked to 2 mL of matrix in the ImmunoPure Protein A IgG Plus Orientation kit (Pierce Biotechnology, Inc. Rockford, IL, USA) by following the manufacturer's instructions. Cotyledonary extracts were collected from 18 cm and 40 cm crown-rump bovine fetuses, dialyzed against 2000 volumes of binding buffer and 100 mg of total protein from each extract was applied separately to each matrix. The columns were washed in binding buffer until the absorbance of the flow-through at 280 nm was at baseline. The bound protein was eluted from the column with 20mM sodium formate, pH 2.8. The eluted proteins were subjected to SDS-PAGE followed by in-gel trypsin digestion, reduction and alkylation of cysteines. The masses of the resulting peptides were then determined by Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry on a Voyager-DE™ PRO Biospectrometry Workstation (Applied Biosystems, Foster City, CA, USA). The monoisotopic masses in the acquired spectra were used for searching against a nonredundant translated mammalian sequence database (NCBIInr) by using the Protein Prospector MS FIT program (<http://prospector.ucsf.edu/>).

The A6 monoclonal antibody exhibited the greatest ability to bind PAG in the placental extracts. The eluted material migrated at three distinct relative molecular mobilities on SDS-PAGE: 55,000, 65,000 and 75,000. Peptide mass fingerprinting revealed that the 75kDa band

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consisted predominantly of PAG7 and PAG6 and the 55kDa band consisted predominantly of PAG16 and PAG4, although weak matches were also observed for PAG17, PAG20 and PAG21 in this band (Table 1). The 65kDa band did not produce many peptides amenable to fingerprinting, but the few that were produced were found to match PAG7.

The yields from the J2 and L4 affinity columns were not as robust as those from the A6 matrix, however, both did permit the purification of a ~70,000 Da protein. The J2-purified protein did not produce many tryptic fragments, but those that were produced matched PAG20 (Table 2). The L4-purified protein was more easily digested and produced numerous masses for fingerprinting. The main PAG isolated from the extracts was PAG21 although other PAGs (PAG17, PAG16 and PAG20) were clearly represented, albeit at lower concentrations (Table 3). The A6 and L4 antibodies bound PAG17 with lower affinity, but the results confirmed the undetectability of this PAG at about two-months post-partum. The major PAGs recognized by each of the monoclonal antibodies (and their relationship to other bovine PAGs) are indicated in the neighbor-joining tree (FIG. 1).

5. The result of the studies demonstrated that PAGs 4, 6, 7, 16, 17, 20 and 21 are undetectable about two-months post-partum and that antibodies for these PAGs may be used in assays for the detection of pregnant bovine animals.

6. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

21 May 2004

Date

Jonathan A. Green

Jonathan A. Green

Received 05/21/2004 13:40 in 02:06 on Line [5] for RH10056 printed 05/21/2004 13:44 * Pg 5/8

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T-032 P.005/008 F-815

Table 1. Assignment of unique digital fragments of PAG-3 affinity purified with the M6 monoclonal antibody.

TSK protein

1. BOS TAURUS (AF020261) progeny-associated phageclone 2.		2. BOS TAURUS (AF020261) progeny-associated phageclone 1.	
Modification	Int. domain	Modification	Int. domain
DB2,300	828,3402	DB2,300	828,3402
727,4150	4,181	727,4150	4,181
727,4119	203,53 (PROLIFERATING)	727,4150	374,578 (PROLIFERATING)
726,4200	37,7632	726,4200	43,2056 (PROLIFERATING)
725,4200	705,4259	725,4200	43,2056 (PROLIFERATING)
871,4680	871,4681	871,4680	243,249 (PROLIFERATING)
1286,5110	1046,5311	1286,5110	202,520 (PROLIFERATING)
1286,5130	1226,5023	1286,5130	157,447 (PROLIFERATING)
1254,6110	1254,6112	1254,6110	137,447 (PROLIFERATING)
1429,6150	1429,6152	1429,6150	30,42 (PROLIFERATING)
1428,7310	1428,7328	1428,7310	30,42 (PROLIFERATING)
1522,7350	1522,7358	1522,7350	310,422 (PROLIFERATING)
1570,8510	1570,8517	1570,8510	102,522 (PROLIFERATING)
2569,2550	2569,2551	2569,2550	144,470 (PROLIFERATING)
2544,1670	2544,1772	2544,1670	216,242 (PROLIFERATING)
2655,3450	2655,3452	2655,3450	194,471 (PROLIFERATING)

1. BOS TAURUS (AF020261) progeny-associated phageclone 10.		2. BOS TAURUS (AF020261) progeny-associated phageclone 9.	
Modification	Int. domain	Modification	Int. domain
1448,8880	1448,7259	1448,8880	18,432 (PROLIFERATING)
1671,7770	1671,5535	1671,7770	40,2802 (PROLIFERATING)
1621,9220	1621,7755	1621,9220	116,225 (PROLIFERATING)
1621,5770	1621,7641	1621,5770	34,9691 (PROLIFERATING)
1664,9000	1664,9008	1664,9000	4,974 (PROLIFERATING)
1770,8200	1759,8082	1770,8200	4,831 (PROLIFERATING)
1820,8200	1820,7675	1820,8200	23,231 (PROLIFERATING)
2012,8250	2012,8257	2012,8250	1,332 (PROLIFERATING)

TSK protein

1. BOS TAURUS (AF020261) progeny-associated phageclone 4.		2. BOS TAURUS (AF020261) progeny-associated phageclone 3.	
Modification	Int. domain	Modification	Int. domain
622,477	842,423	622,477	842,423 (PROLIFERATING)
623,344	833,447	623,344	833,447 (PROLIFERATING)
121,5850	121,5852	121,5850	4,182 (PROLIFERATING)
1257,5850	1232,5731	1257,5850	128,158 (PROLIFERATING)
1275,6862	1378,6582	1275,6862	1,622 (PROLIFERATING)
1428,3407	1430,7114	1428,3407	114,125 (PROLIFERATING)
1780,8449	1780,8450	1780,8449	212,522 (PROLIFERATING)
1772,8450	1772,8450	1772,8450	-4,151 (PROLIFERATING)
1771,8570	1771,8587	1771,8570	148,211 (PROLIFERATING)
1776,8339	1776,8339	1776,8339	213,227 (PROLIFERATING)
2013,0069	2013,0067	2013,0069	184,421 (PROLIFERATING)
2267,185	2267,186	2267,185	141,167 (PROLIFERATING)
2300,2550	2300,2550	2300,2550	250,605 (PROLIFERATING)

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622,300	828,3402	622,300	828,3402
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1522,7350	1522,7358	1522,7350	102,522 (PROLIFERATING)
1570,8510	1570,8517	1570,8510	4,974 (PROLIFERATING)
2569,2550	2569,2551	2569,2550	216,242 (PROLIFERATING)
2544,1670	2544,1772	2544,1670	216,242 (PROLIFERATING)
2655,3450	2655,3452	2655,3450	194,471 (PROLIFERATING)

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2012,8250	2012,8257	2012,8250	1,332 (PROLIFERATING)

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Modification	Int. domain	Modification	Int. domain
622,477	842,423	622,477	842,423 (PROLIFERATING)
623,344	833,447	623,344	833,447 (PROLIFERATING)
121,5850	121,5852	121,5850	4,182 (PROLIFERATING)
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Modification	Int. domain	Modification	Int. domain
622,477	842,423	622,477	842,423 (PROLIFERATING)
623,344	833,447	623,344	833,447 (PROLIFERATING)
121,5850	121,5852	121,5850	4,182 (PROLIFERATING)
1257,5850	1232,5731	1257,5850	128,158 (PROLIFERATING)
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1428,3407	1430,7114	1428,3407	114,125 (PROLIFERATING)
1780,8449	1780,8450	1780,8449	212,522 (PROLIFERATING)
1772,8450	1772,8450	1772,8450	-4,151 (PROLIFERATING)
1771,8570	1771,8587	1771,8570	148,211 (PROLIFERATING)
1776,8339	1776,8339	1776,8339	213,227 (PROLIFERATING)
2013,0069	2013,0067	2013,0069	184,421 (PROLIFERATING)
2267,185	2267,186	2267,185	141,167 (PROLIFERATING)
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1664,9000	1664,9008	1664,9000	4,974 (PROLIFERATING)
1770,8200	1759,8082	1770,8200	4,831 (PROLIFERATING)
1820,8200	1820,7675	1820,8200	23,231 (PROLIFERATING)
2012,8250	2012,8257	2012,8250	1,332 (PROLIFERATING)

1. BOS TAURUS (AF020261) progeny-associated phageclone 4.		2. BOS TAURUS (AF020261) progeny-associated phageclone
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Table 2. Assignment of tryptic digest fragments of PAG affinity purified with the J2 monoclonal antibody.

m/z submitted	M+ matched	Beta form	Start	Peptide Sequence	Modifications
1046.080	1046.0311	52.4526	362-369	(R)LYF8VFPDR(G)	
1687.881	1687.8807	0.2052	29-42	(R)KTL8GKNNMLNNFLK(E)	1PO4
1788.976	1788.9271	26.7724	123-138	(R)LTNKTFGITYGSGRMK(G)	1Met-ox
1794.882	1794.8039	43.5329	217-221	(K)GSVVMFGGVDHRYYK(G)	1PO4
1886.032	1886.0505	-8.7505	3-18	(K)WLVLGLVAFSECIFK(I)	
1927.919	1927.9284	-3.8439	327-342	(R)AYVLKDFTGNCYTTFK(E)	
2221.269	2221.1386	59.1792	117-138	(R)QGSTFRLTNKTFGITYGSGRM(M)	

Table 3. Assignment of tryptic digest fragments of PAG affinity purified with the L4 monoclonal antibody.**BOS TAURUS. (AF182338) pregnancy-associated glycoprotein-21 .**

<u>m/z submitted</u>	<u>M/H+ matched</u>	<u>Delta ppm</u>	<u>Start</u>	<u>Peptide Sequence</u>	<u>Modifications</u>
842.557	842.8484	-12.828	282-288	(K)LVNKDK(L)	
965.513	965.542	-30.0409	327-334	(R)AYILKD(S)(G)	
970.519	970.8413	-23.0012	291-288	(R)KLVNK(D)(L)	
1032.578	1032.5155	60.5738	562-569	(R)YVF(S)VFD(R)(G)	
1088.596	1088.5370	59.4425	127-136	(K)TSITYGSGR(M)	
1178.673	1178.8846	7.1482	327-335	(N)AYILKD(S)(R)(C)	
1201.81	1201.8152	-4.3171	337-345	(R)CYTARKKQR(F)	
1389.729	1389.8985	46.4078	215-229	(R)EGS(Y)MFQGV(DH)(R)(Y)	
1405.714	1405.6534	43.0848	219-226	(R)EGS(Y)MFQGV(DH)(R)(Y)	1Me(Ox)
1733.852	1733.8838	39.4834	113-126	(R)FQHQSSTRPTN(K(T))	
1820.003	1820.9074	82.3487	346-361	(R)F888TETWLLGQAFLR(V)	
1860.005	1860.875	82.8738	215-231	(R)E(Q5)V(MFQGV(DH)(R)(Y)(G)	1Me(Ox)
1969.089	1969.9025	53.2689	232-248	(K)QELNWVPLIEEGDW(SV)(M)	
2153.122	2153.0327	41.4774	30-47	(K)TLSGK(NMLNNFLIKEHGNR(L)	1PO4

Weaker matches were observed for PAG-17, PAG-18 and PAG-20

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